Effects of tip-pruning treatment on source-sink regulation of *Catharanthus roseus* seedlings

GAO Yang, ZHANG Xue-ke, GUO Xiao-rui, SUN Yan-fei, ZU Yuang-gang*

Key Laboratory of Forest Plant Ecology, the Ministry of Education of China, Northeast Forestry University, Harbin 150040, P. R. China

Abstract: Fifty cultivated *Catharanthus roseus* seedlings were selected for tip-pruning treatment and the effects of tip-pruning on seedling growth and source-sink regulation were investigated for revealing physiological mechanisms of plants. The results showed that tip-pruning treatment resulted in obvious inhibition of apical dominance and enhancement of branching numbers. The contents of soluble sugars, acid sucrose invertase activity (AI) had a great change in differently positional leaves of the seedling. The sink strength in tip leaves of seedlings dramatically declined after tip-pruning treatment, while that in the leaves at the middle and bottom of seedlings had no obvious changes. The inhibition of apical dominance of tip leaves of seedlings was caused by the diminished sink strength due to tip-pruning treatment,

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Introduction

The photo-assimilates produced in the source organs are transported into the sink organs mostly in the form of sucrose. Sucrose is released from the sieve elements of the phloem into the apoplast by a sucrose transporter, where it is irreversibly hydrolyzed by acid invertase (Roitsch *et al*. 1996; Gibson 2004). This invertase plays a crucial role in both source-sink regulation and soluble sugar content changes during development and interactions of plants with environmental cues (Roitsch *et al*. 2003). Acid invertase (AI) activity in leaves decreased positively with leaf maturation of plants, with the maximum activity in the youngest leaves. The level of AI activity was not significantly affected in middle leaves and reduced by 46% in the bottom leaves with respect to the invertase activity in the tip leaves of these plants (Xiao *et al*. 2000). In contrast, hexose contents of leaves have been demonstrated to increase with leaf maturation (Yang *et al*. 2004). In addition, exogenous factors such as pruning, wounding and pathogen infection may also alter carbohydrate partitioning and source-sink transition (Ohto *et al*. 2001).

Tip-pruning has been a major artificial way to control plant dominance and improve branching number and seed productions. This treatment has been proposed to be related to source-sink regulation, but the detailed physiological mechanisms involved in this process remains to be uncovered. *Catharanthus roseus* (L.) G. Don is famous for its pharmaceutical utilization and has been cultivated widely, with the application of tip-pruning. For revealing the source-sink relations, AI activity and sugar contents in differently positional leaves of controlled and pruned seedlings were determined in the present research.

Materials and methods

Plant material and treatment

After germinating at the 45th-50th day, the *C. roseus* seedlings were transferred to the seedbed cultivated in the field in Hainan Province. The individuals of 50 seedlings were selected for tip-pruning treatment. The above half part of organs (branch and young leaves) of seedlings were cut at the 50th−60th day after seedlings were transferred. In total of 50 seedlings without any treatment were taken as the control seedlings. There was distinct difference between control and treated seedlings after 40 days in morphology, such as plant height, branch number and leaf size. At this time, seedlings were harvested for further determination of AI activity and sugar contents in different position leaves for seedling.

Determination of AI activity and soluble sugar contents

Activities of soluble acid invertase were measured as described by Hatch and Glasziou (1962). Invertase extract were assayed with an assay buffer containing 50-mM sodium acetate, 15-mM magnesium chloride, and 100-mM Suc. The reaction solutions were incubated for 1.5 h at 32°C and the reaction was terminated immediately after addition of protein extracts. The soluble sugar was extracted in 10 volumes of 85% (v/v) ethanol for 20 min and determined according to Zhou *et al*. (1998). The extracts were subjected to HPLC using an evaporative light-scattering detector, ELSD (Waters, USA) and a column of 250 mm×4.6 mm i.d., 4 μm (Waters, USA).

Results

AI activity variations in differently positional leaves

AI has been described to be a major modulator for source-sink regulation. Immature tissues of sugar cane displayed the highest levels of AI activity, while in maturing and mature tissues AI activity declined rapidly (Leon *et al*. 2003). The displaying patterns of AI activity were considered to direct sucrose to be transported from mature tissues to immature ones as sinks (Roitsch *et al*. 2003). This postulation was confirmed in the present work. It was found that AI activity was highest in the first pair of tip leaves, arriving at 0.189 $g \cdot h^{-1} \cdot g^{-1}$ FW, and decreased significantly during seedling maturating, obtaining the lowest level in the bottom leaves in control seedlings (Fig. 1A).

The second pair of tip leaves in control seedlings were in the earlier phase, required for import of photo-assimilates. While the bottom two pairs of leaves were in the maturing and mature phases, they can export carbohydrates mainly as sucrose to source organs. But in treated seedlings, the characteristics of AI

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Biography: GAO Yang (1982-), female, Master student in Key Laboratory of Forest Plant Ecology, the Ministry of Education of China, Northeast Forestry University, Harbin 150040, P. R. China.

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* Corresponding author: E-mail: zygorl@vip.hl.cn.

activity in differently positional leaves were distinct from that in control seedlings. AI activity of the first pair of tip leaves in treated seedlings was 0.107g·h^{-1}·g^{-1} FW, with the lowest levels, however the AI activity of other three pairs of leaves had 1-fold elevation than that of the first pair of leaves, obtaining about 0.208 g·h^{-1}·g^{-1} FW (Fig. 1B). It was concluded that the displayed pattern of AI in differently positional leaves was inversed in tip-pruned seedlings in contrast to control ones.

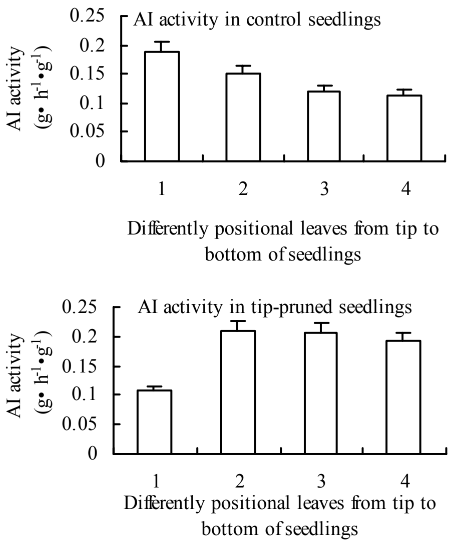

Fig. 1 AI activity in leaves from the tip to bottom of *Catharanthus roseus* seedlings

The number of 1, 2, 3, 4 indicate the first, second, third and fourth pair of leaves from tip to bottom of the seedlings.

Sugar content changes in differently positional leaves

The contents of sucrose, glucose, and fructose in differently positional leaves from the tip to the bottom of the same shoots were checked in control and treated seedlings. The results indicated that hexose levels, including glucose and fructose, in the tip leaves of control seedlings were lowest. With the maturations of leaves, their contents increased. Previous report also showed that the contents of glucose and fructose in leaves increased with leaf age (Quirino *et al*. 2001). It was confirmed in this experiment that fructose reached the peak value of 6 mg·g^{-1} in oldest leaves of control seedlings, 3-fold more than that of young leaves in the tip of seedlings. Sucrose contents in the tip leaves of control seedlings were around 6 mg·g^{-1}, while the third pair of leaves from the tip to the bottom of seedling had the highest contents of sucrose, arriving at 13.5 mg·g^{-1} (Fig. 2a). At the same time, the ratio of sucrose to hexose in the third pair of leaves of control seedlings was also the highest, compared with that of the other leaves. This kind of distribution patterns of sugar suggested that the third pair of leaves was the most active in producing assimilation and capable to supplying it to other leaves.

The treatment of tip-pruning on mainly leaves and young shoots brought about dramatic variations of carbohydrate partitioning in differently positional leaves in the same shoots. The tip leaves were observed to have the highest content of fructose, glucose, and sucrose in unusual way, which were 11.4 mg·g^{-1}, 39.9 mg·g^{-1}, and 23.2 mg·g^{-1}, respectively (Fig. 2b). In contrast to control seedlings, the concentrations of hexose and sucrose were on the opposite in the tip leaves. At the same time, there were no notable gradient in sugar contents among differently positional leaves. These results indicated that the priority of tip leaves as source to receive sucrose was changed and the redundant carbohydrates were translocated to the middle and bottom leaves

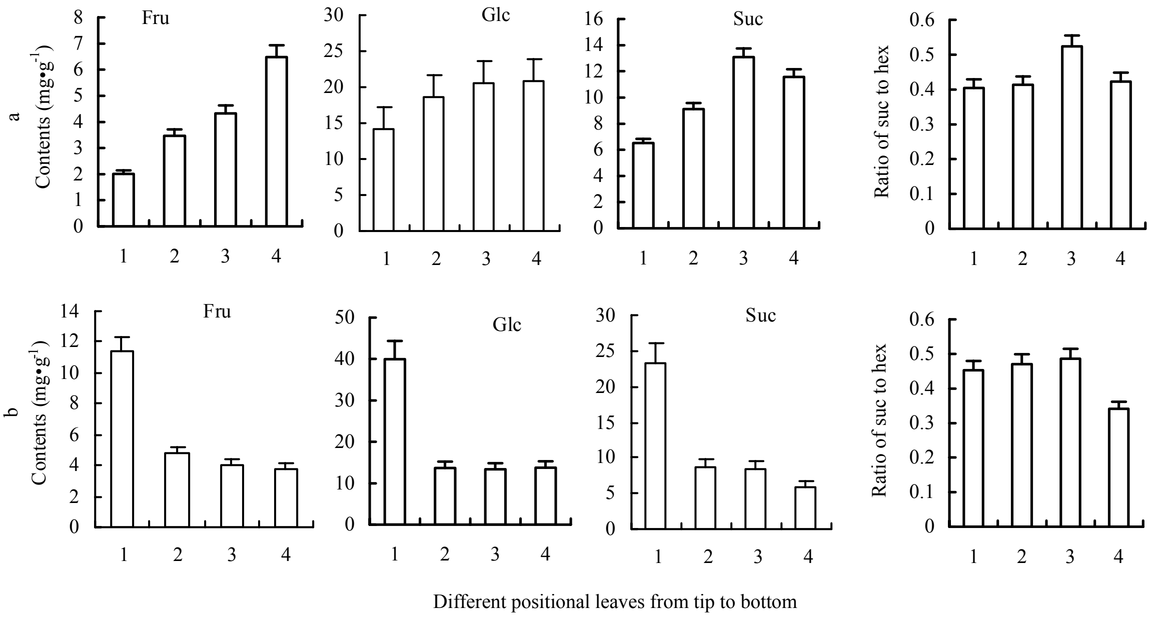

Fig. 2 Sugar contents in differently positional leaves from tip to bottom in *Catharanthus roseus* seedlings

a----sugar contents in control seedlings; b----sugar contents in collected seedlings; number of 1, 2, 3, 4 indicate the first, second, third and fourth pair of leaves from tip to bottom of the seedling.

Discussion

Invertase has been implicated to modulate primary and secondary metabolisms by changes in its activity and subsequently in sucrose and hexose concentrations (Malamy *et al*. 2001). In *Arabidopsis thaliana*, increased leaf carbohydrate export and starch mobilization accompanied with elevated invertase activity in sink leaves are required for apical control and other biological functions (Martin *et al*. 2002). Utilization of sucrose in these sink organs depends on its cleavage into hexoses, and in plants either sucrose synthase or invertase catalyzes this reaction (Paul *et al*. 2001; Paul *et al*. 2003). In the present work, the first pair of tip leaves of control seedlings, as sink leaves, had higher AI activities and lower contents of fructose, glucose and sucrose, corroborating the previous results. The higher level of AI activity may be related to the apical dominancy in control seedlings. But in tip-pruned seedlings, it was found that the sink of tip leaves was greatly reduced, with restrained AI activity and higher levels of fructose, glucose and sucrose, which was likely to be the physiological mechanism for this fostering method.

Conclusions

Source and sink transition indicated by AI activity exchange due to pruning were demonstrated in the present work. The treatment of tip-pruning resulted in decreasing AI activity in the first pair of tip leaves. The decline of AI activity in tip leaves explained that intrinsic sink was inactivated as a result of remove of apical meristem organs.

The changes of AI activities, at the same time, aroused change of sugar metabolism in treated seedlings. The contents of fructose, glucose and sucrose, in the first pair of tip leaves of treated seedlings had about 3-folds elevations. The levels of sugar contents, however, had reduced in other pairs of leaves.

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